

THE BIOCHEMICAL NATURE OF MORPHOGENETIC
PATTERNS IN BLASTOCLADIELLA*

EDWARD C. CANTINO

University of Pennsylvania

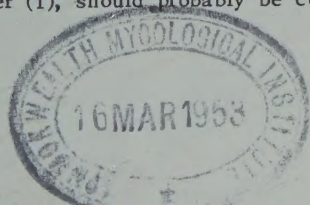
Between gross structure, on the one hand, and the mechanism of its genesis, on the other, lies one of the most fundamental yet essentially unsolved problems in biology. It is now axiomatic that the combined disciplines of genetics and cytology have done much to bridge this hiatus and profoundly influence our concepts of the relation between initiation of structure and its ultimate expression. In most instances, however, there lies between the two extremes a continuous or discontinuous, but none the less superficially descriptive, record of morphogenesis. That such contributions are essential is undisputable, but it seems equally true that further fundamental knowledge of ontogeny will evolve primarily from an understanding of the more deep-seated aspects of growth and development. At one such level of integration lie the causal biochemical transformations which underlie a morphogenetic pattern; in this direction, a start has been made as a result of current studies of the aquatic fungus, *Blastocladiella*.

In this mold, the resistant sporangium (R.S.) germinates readily, provided all inhibitory environmental factors are eliminated; this involves the cracking of a thick, pitted, pigmented wall, and subsequently, discharge of spores through pores formed by deliquescence of papillae on a thin inner membrane. A two-step mechanism is involved (Cantino, 1951) in which cracking and subsequent reactions leading to spore discharge are affected differentially by temperature and anions (figure 1). Some 98 to 100 per cent of the spores derived from R.S. can grow via alternate pathways, one leading to the formation of a thallus bearing, again, an apical thick-walled, pitted, pigmented resistant sporangium, and the other leading to the formation of a thallus bearing a thin-walled colorless "gametangium"¹ with several hyaline papillae. Whether or not the first path is taken is directly related to the presence or absence, respectively, of bicarbonate in the immediate environment of the plant.

It is the purpose of this brief communication to present a unified concept of development in *Blastocladiella*, with particular consideration for the method by which the morphogenetic mechanism, once initiated by such "trigger action," accelerates and tends to go to completion under its own impetus. Because the peptone used at Delft (Cantino, 1951) was unavail-

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¹Evidence, to be published soon, indicates that this structure, tentatively referred to as a "gametangium" in a previous paper (1), should probably be considered a zoosporangium.



able, a new peptone-yeast-glucose-bicarbonate medium ($\text{PYG}^{1/2}\text{B}$),² equally effective for production of R.S. plants, was used for the following experiments. Early in ontogeny, young thalli on small (ca. $\frac{1}{2}$ cm² by $\frac{1}{4}$ cm deep) blocks of $\text{PYG}^{1/2}\text{B}$ will mature into thin-walled plants rather than R.S. plants if the substratum is depleted of most of its bicarbonate by immersion in 5 to 10 cc. of liquid medium PYG. Diffusion rapidly reduces the effective bicarbonate concentration surrounding the plant to ca. 1.3×10^{-4} to 6.5×10^{-5} M. But, beyond a certain critical stage in development, similar reduction of the bicarbonate concentration has no effect, and R.S. plants are formed instead. This critical stage amounts to ca. $\frac{3}{5}$ of the total generation time of $4\frac{1}{2}$ days at 20° C. (figure 2; $\text{PYG}^{1/2}\text{B}$ to PYG curve).

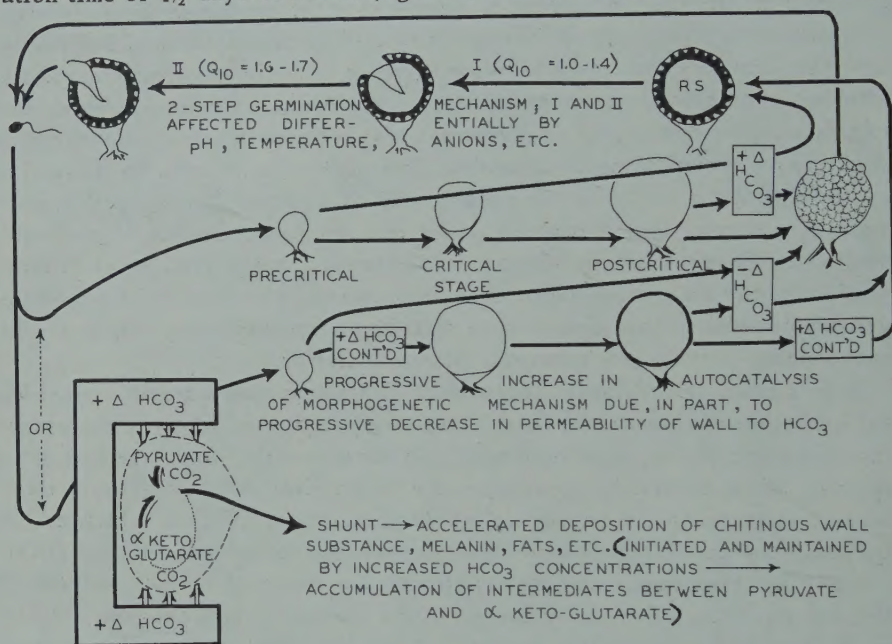


FIGURE 1. The morphogenetic pattern in *Blastocladiella*.

Conversely, germlings on PYG can be induced to form R.S. plants under the influence of bicarbonate only if they are transferred to liquid $\text{PYG}^{1/2}\text{B}$ prior to a similarly critical stage in development. Beyond this point, the mechanism responsible for directing morphogenesis via the R.S. stage be-

²Medium $\text{PYG}^{1/2}\text{B}$: Difco Bacto peptone, 1.25 g.; Difco yeast extract, 1.25 g.; glucose, 3 g.; agar, 20 g.; all dissolved in one liter distilled water containing 1.1×10^{-2} M NaHCO_3 . This medium was further modified by reducing the bicarbonate concentration by one half ($\text{PYG}^{1/4}\text{B}$), by one quarter ($\text{PYG}^{1/8}\text{B}$), etc., or by eliminating the bicarbonate altogether (PYG). On $\text{PYG}^{1/2}\text{B}$, the 1.1×10^{-2} M NaHCO_3 effects production of ca. 100% R.S. plants from R.S. spores, the generation time being about four and one half days at 20° C, at which time the R.S. are immediately viable and capable of germination without a rest period. On the Delft medium, 2.2×10^{-2} M NaHCO_3 was optimum for R.S. production.

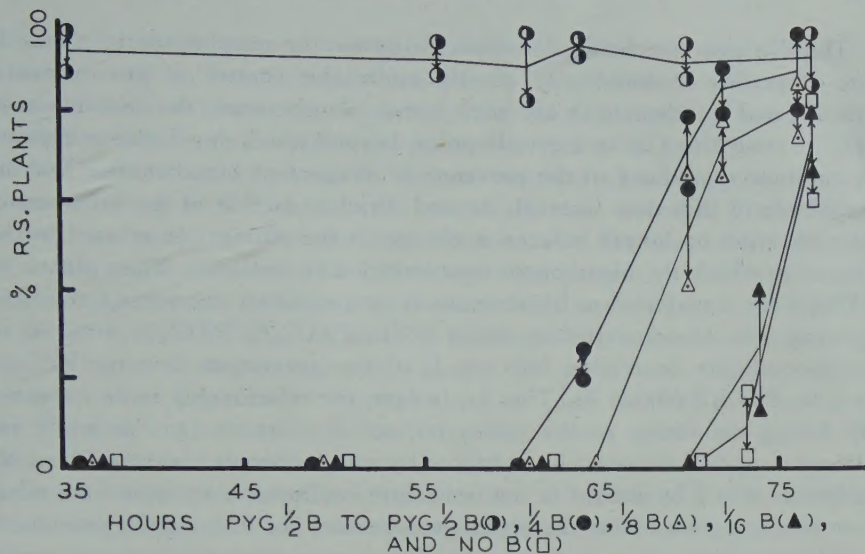


FIGURE 2. (Upper) The relation between reduction of the bicarbonate concentration around plants at different stages of development on PYG $\frac{1}{2}$ B, and the per cent. of such plants ultimately producing resistant sporangia (R.S.). Points represent limits of four or more replicate determinations.

comes inoperative, and again the thin-walled plants are formed. The generation time of the latter, grown on PYG, is approximately 30 to 36 hours at 20° C; the critical point beyond which the morphogenetic pattern can no longer be reversed by addition of bicarbonate is again ca. $\frac{3}{5}$ of the generation time (figure 3).

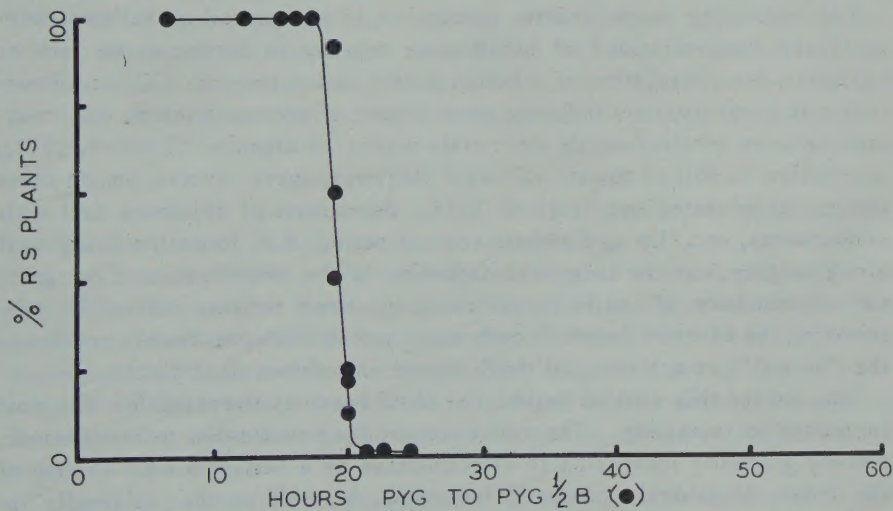


FIGURE 3. (Lower) The relation between increase in bicarbonate concentration around plants at different stages of development on PYG, and the per cent. of such plants ultimately producing resistant sporangia (R.S.).

The "trigger mechanism" which initiates the morphogenetic shunt in one direction or another is clearly under the control of environmental stimuli, and bicarbonate is one such factor. Furthermore, the stimulus need only be maintained up to a certain point, beyond which development appears to continue regardless of the presence or absence of bicarbonate. But the magnitude of this time interval, beyond which reduction of the bicarbonate concentration no longer induces a change in the pattern, is related to the degree to which the bicarbonate concentration is reduced. When plants on $\text{PYG}\frac{1}{2}\text{B}$ are transferred to bicarbonate at progressively increasing strength, starting with bicarbonate-free media (PYG , $\text{PYG}\frac{1}{16}\text{B}$, $\text{PYG}\frac{1}{8}\text{B}$, etc.), it is correspondingly decreased from ca. $\frac{3}{5}$ of the generation time for PYG to zero for $\text{PYG}\frac{1}{2}\text{B}$ (figure 2). This is, in fact, the relationship to be expected if, during maturation of the plant, its cell membranes (and/or wall) exhibited a gradual decrease in permeability to bicarbonate; less and less bicarbonate would be needed in the immediate environment to ensure the minimum concentration within the organism necessary for R.S. morphogenesis.

Other evidence, too, suggests that a resistant sporangium possesses a distinctly different degree of permeability to various solutes than does a thin-walled plant. Thalli stained with neutral red at different stages of development and subsequently transferred to liquid $\text{PYG}\frac{1}{2}\text{B}$ containing neutral red, swiftly undergo a color change from red to yellow, presumably due to inward diffusion of bicarbonate. But the rapidity with which the transition takes place decreases with increasing age of the developing R.S. plants. These results are consistent with and offer further evidence for the notion that during the ontogeny of R.S. plants, permeability progressively decreases.

The underlying morphogenetic mechanism is interpreted as follows: with increased concentrations of bicarbonate tending to decrease the rate of oxidative decarboxylation of α -ketoglutarate and accelerate CO_2 condensation with pyruvate, thus inducing some degree of accumulation of intermediates between α -ketoglutarate and citrate within the organism (Cantino, 1951), metabolism is shifted toward alternate pathways; these involve, among other things, accelerated synthesis of lipids, deposition of chitinous cell wall constituents, etc. Up to a certain critical period, R.S. formation being well along judging from the increased darkening of the protoplasm and the gradual accumulation of lipid-like globules, the trend remains reversible. By removing the external factor (bicarbonate), metabolism presumably reverts to the "normal"; at any rate, all thalli mature into thin-walled plants.

But, beyond this critical period, the trend becomes irreversible. The wall increases in thickness. The cell becomes less permeable to bicarbonate, thereby gradually increasing in effectiveness as a barrier toward escape of the latter; bicarbonate produced internally, as well as that originally introduced from outside, tends to be retained, with the result that increasingly high concentrations of it remain within the organism. It is ensured, through reduction of the rate of decarboxylation of α -ketoglutarate, that a normal flow of intermediates via the energy-yielding machinery of the tricarbox-

lytic acid cycle is not and will not be initiated. There is thus produced a structure with an abundant supply of reserve "fat," a thick wall, and a supposedly low rate of general metabolic activity—a resistant sporangium well suited to withstand adverse environmental conditions (figure 1).

The bulk of our knowledge of dynamic aspects of morphogenesis in the plant kingdom has been derived from studies of vascular plants among which, according to Wetmore (cf. Wetmore and Wardlow, 1951, p. 288), "...the genetically controlled, developmental pattern is stable enough to withstand extensive variations in the nutritional environment." The growth habit of *Blastocladiella*, an aquatic Phycomycete, cannot be compared to that of higher plants, wherein, for example, an apical multicellular meristem controls shoot organization by regulating, in some fashion, the phyllotaxis of leaf and bud primordia. In fact, the determinate growth system of this fungus is as much in striking contrast to the indeterminate habit of vascular plants as development in the latter is to that in vertebrate animals. On the other hand, ontogenesis of the coenocytic thallus with its single septum in *Blastocladiella* is far removed from the development of the multicellular system of a vertebrate, with its gastrulation and other complex phenomena. Perhaps, however, observations and conclusions derived from the study of this water fungus can, in part, be reconciled with and integrated into the concepts of morphogenesis evolved from extensive investigations of the vertebrates and invertebrates (cf. for example, Weiss, 1939). In brief, at an early stage in ontogeny, the differentiation potency of the simple thallus of *Blastocladiella* is not limited to the one course indicated by its prospective fate (under ordinary conditions); that is, it is pluripotent. Selection of an alternative potency may be induced initially by an external factor such as bicarbonate, and subsequent unfolding of the process is controlled from within; determination must occur at this time, or later, but not before. Beyond a critical stage in development, however, the original prospective fate has become fixed and final, and cannot be altered by adding bicarbonate.

CONCLUSION

In conclusion, in *Blastocladiella*, this phase of morphogenesis (figure 4):
(a) is *initiated* by an *external* factor, bicarbonate.

(b) once initiated, is *sustained* only so long as the external factor is maintained for a certain duration; that is, until the newly developed morphogenetic pattern has achieved a certain degree of maturity. This involves a concomitant shift in metabolism leading to the gradual production of alternate metabolic products on a molecular level and is simultaneously reflected in the initiation of a different structure at the gross morphological level.

(c) having passed the critical stage, is *completed* irrespective of the presence or absence of the external factor because *internal* factors (among them, a deranged metabolism and rapidly decreasing permeability) now ensure an overall unidirectional flow from the metabolic machine under the complex of their own gathering intensity; the system (increased retention of

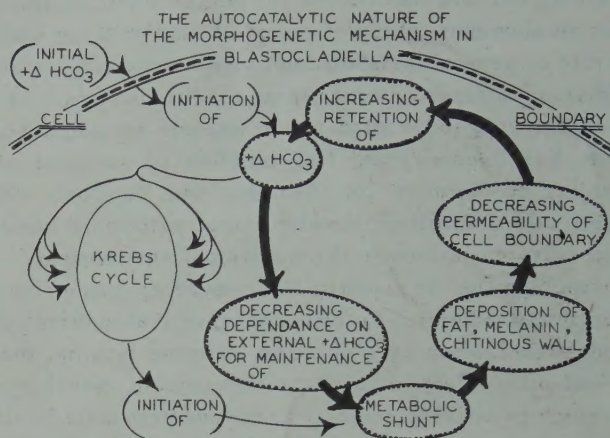


FIGURE 4. The autocatalytic nature of morphogenesis in *Blastocladia*.

$\text{HCO}_3 \rightarrow$ increased flow of metabolites along new path $\rightarrow$ gradual thickening of wall and decrease in permeability $\rightarrow$ increased retention of HCO_3 , etc.) can best be likened to a kind of internal autocatalysis which guarantees the ultimate formation of a resistant sporangium.

LITERATURE CITED

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